

Attorney Docket No.: DC-0199
Inventors: Cheung et al.
Serial No.: 10/043,539
Filing Date: January 11, 2002
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Attached hereto is a marked up version of the changes made to the specification by the current amendment. The marked up version of the changes is captioned "Version With Markings To Show Changes Made".

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 6, page 30 has been amended as follows:

Cloning and sequence analysis of the *sarR* gene. To clone the gene encoding SarR, we blotted the ~12 kDa protein onto a PVDF membrane for N-terminal sequencing. The first 14 amino acids were X(K)IND(I)NDLVNA(S/T)F, (~~Seq~~ SEQ. ID NO.:8) with X being any unknown residue while those residues in parenthesis carried a putative assignment. In searching the databank of the partially released *S. aureus* genome (www.tiger.org), we obtained a partial ORF of 47 amino acid sequence that corresponds to the N-terminal sequence of the ~12 kDa protein. By using two degenerate oligonucleotides of 30-nt each, a 141-bp fragment was amplified to probe a chromosomal digest of *S. aureus* strain RN6390, thus allowing identification of a ~4 kb *Cla*I hybridizing fragment. A plasmid DNA library containing ~3-5 kb *Cla*I fragments constructed in pACYC177 (26) was then screened with the 141-bp PCR-generated probe. A positive clone (pALC1361) yielding a ~4-kb insert at the *Cla*I site of pACYC177 vector was identified. In determining the sequence of the insert, and comparing the insert sequence with that of the 141-bp probe, the DNA sequence of the putative gene *sarR* was obtained (Fig. 1B) (GenBank accession #AF207701). The predicted SarR protein contains 115 amino acids, with a predominance of charged residues (34%) and a predicted molecular size of

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13,689 daltons. The *sarR* gene has a putative Shine Dalgarno sequence (AGGAGTGG) (SEQ. ID NO:9) lying 7-bp upstream of the translation start, with typical initiation (ATG) (SEQ. ID NO:33) and termination codons (TAA) (SEQ. ID NO:34). To ascertain the transcription start site and the putative promoter boxes, the 5'-end of the *sarR* transcript was mapped by primer extension, using an internal primer of the non-coding strand positioned near the N-terminus of the *sarR* coding region. The transcription initiation site is located 88-bp upstream of the translation start, thereby allowing identification of the putative -10 and -35 promoter boxes as TAGAAT (~~SEQ. ID. No. 10~~ SEQ. ID NO:10) and TTACCG (~~SEQ. ID. No. 11~~ SEQ. ID NO:11), respectively (Fig. 1B).